31, 146 (1957).

8) Ogita, Z. : Inform. Cir. Resistance Problem, W. H. O. 12, 4 (1958).

9) Oshima, C. and T. Hiroyoshi : Botyu-Kagaku 21, 65 (1956).

10) Tsukamoto, M. : Botyu-Kagaku 20, 73 (1955).

11) Tsukamoto, M. and M. Ogaki : Botyu-Kagaku 18, 39 (1953).

12) Tsukamoto, M. and M. Ogaki : Botyu-Kagaku 19, 25 (1954).

13) Tsukamoto, M. , M. Ogaki and H. Kikkawa: Proc. Intern. Genet. Symposia 442 (1956).

14) Tsukamoto, M. and T. Hiroyoshi: Botyu-Kagaku 21, 71 (1956).

15) Wichmand, H. : Working paper 8 Add. 1, Expert committee on Insecticide, W. H. O. (1956).

The Effects of Rotenone and Its Derivatives on the Respiration of Brain in Guinea Pig.

36. モルモット脳の呼吸に及ぼすロテノーンおよびその誘導体の影響　深見順一　（東京大学農学部 害虫学研究室）・富沢長次郎（農林省農業技術研究所）33. 9. 26　受理

ロテノーンは昆虫体内で神経および筋肉の細胞呼吸を抑制し，死に至らしめるが，その細胞呼吸抑制の一部はグルタミン酸脱水素酵素の抑制によるものである⁵⁾．しかし昆虫におけるグルタミン酸脱水素酵素の細胞呼吸における役割は不明である．そこで筆者らはグルタミン酸脱水素酵素の役割が細胞呼吸においてかなり理解されているモルモットの脳を使用してロテノーンの影響を検討した．モルモット脳ホモジェネートおよびミトコンドリアに基質無添加，コハク酸およびグルタミン酸を添加した場合の酸素消費に対するロテノーンおよびその誘導体の影響を調べたところ，その阻害の態度は昆虫の場合と同じ傾向を示した．

The authors have concluded in the previous reports that rotenone inhibits the respiration of nerve and muscle in insects, and the inhibition of the respiratory metabolism is partly due to the inhibition of the glutamic dehydrogenase activity[4,5]. It was also found in rotenone and its derivatives that a close correlation exists between the *in vivo* toxicity against insects and the degree of the inhibition of glutamic dehydrogenase in insect muscle[6].

Although the role of glutamic dehydrogenase in cellular respiration is not known in insects, it is more fully demonstrated in the brain of mammals. The present study was therefore undertaken with the purpose of determining whether rotenone and its derivatives inhibited the homogenate respiration with or without succinate and the glutamic dehydrogenase activity from the brain of the guinea pig.

Materials and Methods

All animals used were male guinea pigs weighing approximately 250 g.

Measurement of oxygen consumption of brain homogenate : Experiments were carried out using the Warburg manometers at 37°C. The animals were killed by decapitation. A brain (1.5 g) which contained cerebrum, cerebellum and medulla oblongata was homogenized with five times as much as volume of phosphate buffer (1/15 M, pH 7.4) as the brain and filtered through gauze. These procedures were carried out at 10°C. Flask contents were as follows : 1/15 M phosphate buffer (pH 7.4) 1.2 ml, substrate 0.4 ml (final concentration, 0.033 M), homogenate 1.3 ml, distilled water 0.7 ml or distilled water 0.3 ml plus inhibitor 0.4 ml.

Measurement of oxygen consumption by the

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mitochondrial fraction of brain: Experiments were carried out using the Warburg manometers at 37°. The brains similar to those used in the homogenate experiments were employed as the source of mitochondrial fraction. The mitochondrial fraction was isolated by a slight modification of the technique already reported[5]. About 6 g of the brains obtained from 4 male guinea pigs were homogenized with 50 ml of a solution containing 0.02 M substrate, 1/15 M phosphate buffer (pH 7.2) and 0.25 M sucrose. The resulting brei was filtrated through three layers of surgical gauze. On centrifuging the filtrate for 5 minutes at 500×g, the precipitate was discarded. The supernatant was centrifuged for 15 minutes at 10000×g. The precipitate was resuspended in 13 ml of a solution containing 0.02 M substrate, 0.02 M phosphate buffer (pH 7.2) and 0.9 % KCl, and centrifuged for 5 minutes at 8000×g. The precipitate was resuspended in 5 ml of a solution containing 0.02 M substrate, 1/50 M phosphate buffer (pH 7.2), 0.9% KCl and 10^{-5} M cytochrome c, and the suspension was taken as the mitochondrial fraction for the measurements of oxygen uptake. The final pH of the suspension was 7.1-7.2. The pH of the brain homogenate was adjusted over 7.0 by adding 0.2 M tris (hydroxymethyl) aminomethane buffer (pH 9.0) during the isolation of the mitochondrial fraction. The whole procedures were carried out at 0 to 3°C.

Rotenoids tested were as follow: rotenone, rotenolone-I, dihydro-rotenone and dehydro-rotenone. The chemical structures of these compounds were shown in the previous report[6].

Results

At first, the *in vitro* effect of rotenone on the oxygen uptake of the homogenate with or without succinate were examined. The results are shown in Table 1. Rotenone inhibited the endogenous oxygen uptake, but did not inhibit the oxygen uptake in the presence of succinate. Similar experiments using the insect homogenate[3] are also shown in Table 1 for comparison. It can be seen that there is no substantial difference between insect and mammal.

Next, the *in vitro* effects of rotenone and its

Table 1. *In vitro* effects of rotenone on the O_2 uptake of the homogenates of insect muscle and guinea pig brain with or without succinate. Final concentration of rotenone, 5×10^{-5} M.

	Per cent inhibition of O_2 uptake	
	Guinea pig brain	Insect muscle[3]
Endogenous	79	54
With succinate	6	9

Table 2. *In vitro* effects of rotenone and its derivatives on the O_2 uptake of the mitochondrial fractions of insect muscle and guinea pig brain in the presence of *l*-glutamate.

Compound	Final concentration (M)	Per cent inhibition of O_2 uptake	
		Guinea pig brain	Insect[6] muscle
Rotenone	10^{-5} 10^{-6}	64 64	85 82
Dihydro-rotenone	10^{-5}	59	82
Rotenolone-I	10^{-5}	34	75
Rotenolone-II	10^{-5}	—	6
Dehydro-rotenone	10^{-5}	+18	14

derivatives on the oxygen uptake of the mitochondrial fraction in the presence of *l*-glutamate were examined. The results are shown in Table 2. Rotenone, dihydro-rotenone and rotenolone-I inhibited the glutamic dehydrogenase, but dehydro-rotenone did not. Similar experiments using insect muscle[6] are also shown in that table for comparison. It can be seen that a similar tendency holds for both insect and mammal.

Discussion

The lethal dosages of rotenone, dihydro-rotenone and dehydro-rotenone in guinea pig are of the same order as those in insects as shown in Table 3.

It has been shown that in the mammalian brain there is a close correlation between the content of *l*-glutamate and nerve function, and that *l*-glutamate is the only amino acid to be oxidized in the brain and is found in the brain in greater amount than in any other organs[9,13]. Since a change in the ratio *l*-glutamate to α-ketoglutarate is usually followed a by change in proportion of

Table 3. Relative toxicities of rotenone and its derivatives.

Compound	Silk[10] worm	Army[7] worm	Azuki* bean weevil	Guinea[1] pig
Rotenone	100	100	100	100
Dihydro-rotenone	33	—	70	40
Rotenolone-I	—	—	50	—
Rotenolone-II	—	—	0	—
Dehydro-rotenone	0.15	0	0	0

* Fukami *et al.* (unpublished data)

TCA cycle constituents, *l*-glutamic dehydrogenase which controls this ratio might play an important role in the regulation of respiration[9]. Takagaki *et al.*[11] found that the formation of ammonia in the brain cartex depends upon the oxidative deamination of *l*-glutamic acid, and that more than half of endogenous oxygen consumption is related to a decrease of *l*-glutamic acid content. These facts point out that the important substrate in the endogenous oxygen consumption of the brain cortex is glutamic acid. Therefore, it can be supported that the death of guinea pig by rotenone intoxication is related to the inhibition of cellular respiration in the brain, and that this is mainly due to the inhibition of *l*-glutamic dehydrogenase.

On the other hand there is few available evidence indicating the correlation between *l*-glutamate and nerve function in insects. The authors found that the content of *l*-glutamate in the cockroach nerve cord is great as compared with other free amino acids and that *l*-glutamate is utilized very much in the nerve cord[5]. Tanaka *et al.*[15] also pointed out the high amount of *l*-glutamate in the ganglia of the silkmoth and of the silkworm.

The results obtained so far on the effects of rotenone intoxication on cellular respiration together with the role of *l*-glutamate are summarized in Table 4. It is probable that *l*-glutamic dehydrogenase plays an important role in the regulation of respiration in insect as well as in mammalian brain, and hence the inhibition of *l*-glutamic dehydrogenase plays a major part in respiratory inhibition by rotenone in insect nerve as well as in mammalian brain.

Summary

The effects of rotenone and its derivatives on cellular respiration of the brain of guinea pig

Table 4. Comparison of respiratory metabolism and degree of its inhibition by rotenoides between mammal and insect.

	Insect		Mammal Brain	Reference
	Nerve	Muscle		
Physiological function of *l*-glutamate	Unknown	Unknown	Almost known	(11)
Utilization of *l*-glutamate	+++	+++	+++	(5), (9), (13)
The content of *l*-glutamate	+++	+	+++	(2), (5), (9), (12), (13)
Inhibition by rotenone (*in vitro*)				
Endogenous,　　　　T or H	+	+	+	(3), (4), Present work
Succinate oxidation,　T or H	—	—	—	(3), Present work
M		—		(5)
L-glutamate oxidation,　T	+			(5)
M		+	+	(5), Present work
The correlation between the degree of inhibition of *l*-glutamate oxidation and the relative toxicities by rotenone and its derivatives		Correlation exists	Correlation exists	(6), Present work

T : TTC reaction, H : Homogenate, M : Mitochondrial fraction, + : Presence, — : Absence

have been examined.

The oxygen uptake of the homogenate without substrate was inhibited by rotenone, but it was not inhibited when succinate was added.

When l-glutamate was added as substrate, the oxygen uptake of the mitochondrial fraction of brain was seriously inhibited by rotenone.

Dihydro-rotenone and rotenolone-I were effective in depressing l-glutamate oxidation, but dehydro-rotenone was ineffective.

These results are the same as those obtained in insects which have been reported previously.

References

1) Ambrose, A. M. and H. B. Haag : Ind. Eng. Chem. **29**, 429 (1937).

2) Braustein, A. E. : Advances in Protein Chemistry **3**, 1 (1947).

3) Fukami, J. : Japan. J. Appl. Zool. **19**, 148 (1955).

4) Fukami, J. : Botyu-Kagaku **21**, 122 (1956).

5) Fukami, J. and C. Tomizawa : Botyu-Kagaku **21**, 128 (1956).

6) Fukami, J. and C. Tomizawa : Botyu-Kagaku **23**, 1 (1958).

7) Haller, H. L., L. D. Goodhue and H. A. Jones : Chemical Review **30**, 33 (1942).

8) Nakatsugawa, T., J. Fukami, C. Tomizawa, and H. Ishikura : Abstract of the annual meeting of Japan. Soc. Appl. Entomol. and Zool., Tokyo (1958).

9) Okamoto, A. : Shinkei Kagaku (Neurochemistry), Igaku Shoin, Tokyo (1952).

10) Shepard, H. H. and F. L. Campbell : J. Econ. Entomol. **25**, 142 (1932).

11) Takagaki, G., S. Hirano, and Y. Tsukada : Arch. Biochem. Biophy. **68**, 196 (1957).

12) Tanaka, I., K. Takeda, and T. Takehana : Abstract of the annual meeting of Japan. Soc. Sericul. Soc., Tokyo (1957).

13) Tukada, U. : Seitai no Kagaku (Medical Science) **5**, 107 (1953).

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37. Dialkyl dithiophosphoric acid 及び dialkyl hydrogen phosphite のオレフィン化合物への附加 有機燐化合物の研究 第4報 永江祐治・渡辺智夫・大熊一男(東亜農薬株式会社 農薬研究所) 33. 10. 6 受理

殺虫作用を検討する目的を以て、dialkyl dithiophosphoric acid 及び dialkyl hydrogen phosphite のオレフィン化合物への附加反応について研究した。Dialkyl dithiophosphoric acid はアクリロニトリル (VI)、シクロペンタジェン、ジシクロペンタジェン (X) に附加して夫々対応する dithiophosphate (V, VII, VIII, IX) を与えた。同様の附加反応を dialkyl hydrogen phosphite について試み、VI よりは対応する phosphonate (XI, XII) を得たが、X よりは成績体を得ることができなかった。

Mel'nikov et al[1]. prepared many dialkyl dithiophosphoric acid esters by addition of dialkyl dithiophosphoric acids to olefinic compounds. Gar et al[2]. evaluated their biological properties, and found that dithiophosphates derived from acrylonitrile or diethyl fumarate were the most effective. Dithiophosphate obtained from diethyl fumarate and dimethyl dithiophosphoric acid, is an excellent insecticide known as the trade name

"Malathion"[3,4].

Gar et al. tested biological properties of dithiophosphates only against *Calandra oryzae*. It seemed to us to be interesting if dialkyl β-cyanoethyl dithiophosphates, prepared by cyanoethylation of dialkyl dithiophosphoric acids, would have the similar biological properties to those of Malathion, which had low toxicity to mammals and specific effects against aphids, mites and green rice leafhoppers. Hence we prepared some dithiophosphates derived from acrylonitrile, espe-

*　Part III : Botyu-Kagaku, **23**, 115 (1958).

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